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Environmental DNA is an effective tool to track recolonizing migratory fish following large-scale dam removal

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Abstract

Environmental DNA (eDNA) has emerged as a potentially powerful tool for use in conservation and resource management, including for tracking the recolonization dynamics of fish populations. We used eDNA to assess the effectiveness of dam removal to restore fish passage on the Elwha River in Washington State (USA). Using a suite of 11 species-specific eDNA polymerase chain reaction (PCR) assays, we showed that most targeted anadromous species (five Pacific Salmon species and Pacific Lamprey) were able to pass upstream of both former dam sites. Multiscale occupancy modeling showed that the timing and spatial extent of recolonization differed among species during the four years of post-dam removal monitoring. More abundant species like Chinook Salmon and Coho Salmon migrated farther into the upper portions of the watershed than less abundant species like Pink Salmon and Chum Salmon. Sampling also allowed assessment of potamodromous fish species. Bull Trout and Rainbow Trout, ubiquitous species in the watershed, were detected at all sampling locations. Environmental DNA from Brook Trout, a non-native species isolated between the dams prior to dam removal, was detected downstream of Elwha dam but rarely upstream of the Glines Canyon Dam suggested that the species has not expanded its range appreciably in the watershed following dam removal. We found that eDNA was an effective tool to assess the response of fish populations to large-scale dam removal on the Elwha River.

KEYWORDS
anadromous, Bull Trout, dam removal, eDNA, Elwha River, Lamprey, Pacific Salmon, recolonization

1 | INTRODUCTION

Structures such as culverts, dams, and weirs that prevent or alter the upstream and downstream movement of migratory organisms can negatively affect fish populations in a river system. These effects include disruption of spawning migrations, reduction or elimination of habitat access, degradation of downstream habitat (e.g., loss of fluvial transport of sediment, wood, and nutrients), alteration of temperature and flow regimes, fragmentation of populations and their gene flow, and reductions in population productivity (Baxter, 1977; Lucas & Baras, 2001; National Research Council, 1996; Yamamoto,

Morita, Koizumi, & Maekawa, 2004). Restoring fish passage through fish bypass structures or the removal of barriers on rivers can return longitudinal connectivity to migrating fish populations, allowing for restored population connectivity, gene flow, and access to habitat (Nunn & Cowx, 2012). The practice of barrier removal has been increasingly used to restore and conserve fish species (Kemp & O'Hanley, 2010). In many cases, the restoration of fish passage is a primary goal of barrier removal projects (Bernhardt et al., 2005; Doyle, Harbor, & Stanley, 2003; Stanley & Doyle, 2003), including dam removal projects that are becoming more common in the face of aging infrastructure, safety concerns, and a desire to restore the structure and function of river systems (Bellmore et al., 2019; Foley et al., 2017; O'Connor, Duda, & Grant, 2015).

Once longitudinal connectivity is restored, many fish species resume historical migration patterns past the former barrier and recolonize upper reaches of watersheds (Hitt, Eyler, & Woodford, 2012; Pess, Quinn, Gephard, & Saunders, 2014). Fishes that are diadromous, migrating between marine and freshwater environments, are well suited to exploit opportunities for recolonization. For example, diadromous species like Pink Salmon (*Oncorhynchus gorbuscha*; Pess, Hilborn, Kloehn, Quinn, & Bradford, 2012), Coho Salmon (*O. kitsuch*; Anderson & Quinn, 2007; Kiffney et al., 2009), Steelhead (*O. mykiss*; Allen et al., 2016), American Eel (*Anguilla rostratta*; Hitt et al., 2012), and American Shad (*Alosa sapidissima*; Burdick & Hightower, 2006) have successfully recolonized upstream areas following restored passage through installation of fish bypass or dam removal. These species move into and exploit newly accessible habitat over time. The degree to which they expand their range and increase their population productivity varies depending upon extrinsic factors like distance to source populations and the amount and condition of upstream habitats, as well as intrinsic factors such as population size and retention of suitable life-history characteristics for exploiting restored habitats (Pess et al., 2014).

Collecting empirical data on species-specific recolonization and redistribution patterns following barrier removal is necessary for evaluating the effectiveness of such projects in restoring fish populations (Clark, Roni, Keeton, & Pess, 2019). Determining the spatial and temporal dynamics of recolonization, for example, requires data on the timing and extent of how far fish are migrating into newly available habitat. Availability of these data depends in large part upon the watershed setting, the taxa of interest, and the availability of resources to conduct fieldwork. The complexity of research questions also drives data collection requirements and the techniques employed. At a minimum, knowing whether and when fish arrive into habitats upstream of former barriers is vital for documenting and assessing fish passage. Additional data on the spatial extent of recolonization, the diversity of habitats utilized by colonizing individuals (e.g., mainstem vs. tributary use), the origin of recolonizers, the success of spawning and rearing in newly opened habitats, and population-level responses (e.g., population growth rate) allow for more detailed descriptions of the mechanics and outcomes of recolonization. In most cases, these more complex questions also require a variety of labor-intensive fisheries techniques to gather the data—such

as spawner surveys or sonar enumeration for adult return data and smolt traps or pit tag arrays to estimate juvenile outmigration and survival (e.g., Zimmerman, Kinsel, Beamer, Connor, & Pflug, 2015).

In this paper, we describe a study to track the recolonization of anadromous Pacific salmon and lamprey and the redistribution of resident fish species into newly accessible habitat in the Elwha River, following the removal of two high-head, long-standing dams that lacked fish passage facilities. The largest dam removal project to date was completed on the Elwha River, Washington, USA, over a 3-year period (2011–2014), with the primary goal being restoration of fish passage and rebuilding of anadromous salmon populations that spawn in the river, which had been significantly impacted by the dams (Duda, Freilich, & Schreiner, 2008; Pess, McHenry, Beechie, & Davies, 2008; Wunderlich, Winter, & Meyer, 1994). Although using a suite of fisheries techniques was possible in the easily accessible portions of the river (i.e., areas with road access, hereafter termed "front-country"), approximately 70% of the watershed occurs in roadless areas of Olympic National Park (ONP) that are protected as wilderness ("backcountry"). Use of some front-country sampling techniques, especially those requiring regular equipment maintenance (e.g., sonar) or unwieldy, logistically intensive equipment requiring daily labor (e.g., smolt traps), was prohibitively challenging in the roadless backcountry regions of the National Park where some of the species were expected to recolonize (Brenkman et al., 2008; Pess et al., 2008).

We identified the use of environmental DNA (eDNA) as a cost-effective solution to document fish recolonization in logistically challenging portions of the Elwha watershed (i.e., roadless backcountry areas which constitute most of the watershed area) after dam removal. The use of eDNA has rapidly grown since the earliest studies describing the ability to detect targeted organismal DNA from environmental water samples (Ficetola, Miaud, Pompanon, & Taberlet, 2008). Today, there are multiple techniques for both targeted, species-specific approaches (e.g., PCR, qPCR, ddPCR) and community-level profiles based on sequence data (Rees, Maddison, Middleditch, Patmore, & Gough, 2014). Sampling for eDNA has occurred for a wide array of targeted organisms in aquatic environments including rivers, lakes, ponds, and the ocean (Deiner, Fronhofer, Mächler, Walser, & Altermatt, 2016; Doi et al., 2017; Goldberg, Pilliod, Arkle, & Waits, 2011; Hänfling et al., 2016; Kamoroff & Goldberg, 2018; Pilliod, Goldberg, Arkle, & Waits, 2014; Rees et al., 2014). Given the broad application and sensitivity of eDNA methods for species detection, the use of eDNA to track the presence or nondetection of targeted fish species following dam removal was an ideal practical application of this technique.

Our goals were to (a) apply a collection of 11 species-specific molecular markers (of which eight were developed herein) for use in real-time PCR amplification of eDNA to detect the presence of resident and migratory fish species (Table 1) occurring in the Elwha River following dam removal. Due to the nature of the study watershed, we needed to develop field protocols for consistent use across front-country and backcountry settings ranging over 56 river kilometers (rkm) of the Elwha River, including tributaries; and (b) examine

TABLE 1 Fish species targeted for environmental DNA (eDNA) sampling following dam removal in the Elwha River

Common name (Species)	Life-history type	Ave. length ± SD (cm) N (years sampled)	Ave. adult age (range) N (years sampled)
Chinook Salmon (O. tshawytscha)	Anadromous	82 ± 10 *1,616 (2014–2019)*	4 (3–6) *1,616 (2014–2019)*
Chum Salmon (*O. keta*)	Anadromous	70 ± 7 *17 (2015–2019)*	4 (3–5)[b]
Coho Salmon (*O. kisutch*)	Anadromous	56 ± 12 *236 (2013–2019)*	3 (2–3)[b]
Pink Salmon (*O. gorbuscha*)	Anadromous	51 ± 5 *700 (2010–19)*	2[a]
Sockeye Salmon (*O. nerka*)	Anadromous	58 ± 6 *72 (2014–2019)*	4 (3–6)[b]
Rainbow Trout/Steelhead (*O. mykiss*)	Resident/Migratory	25 ± 12/66 ± 10 *403/1057 (2010–2019)*	Unknown/4 (4–6)
Coastal Cutthroat (O. clarkii clarkii)	Resident/Migratory	Unknown	Unknown
Bull Trout (*Salvelinus confluentus*)	Resident/Anadromous	48 ± 7 *250 (2010–2019)*	4 (3–7)
Brook Trout (*S. fontinalis*)	Resident/Non-native	Unknown	Unknown
Pacific Lamprey (Entosphenus tridentatus)	Anadromous	60 ± 3 *19 (2018)*	Unknown
Lampetra spp.	Resident/Migratory	Unknown	Unknown

Note: All Pacific salmon are in the genus *Oncorhynchus*. Data on average length and lifespan compiled from existing field studies, including spawner and carcass surveys, hatchery records, and weir (WDFW, LEKT, FWS, NOAA) or estimated from the literature.

[a]Based on typical Pink Salmon in Puget Sound, Washington. All individuals are 2 years old.

[b]From Groot and Margolis (1991).

seasonal and annual patterns of eDNA detections as a proxy for targeted fish species presence at sites throughout the watershed, focusing on the spatial extent and temporal dynamics at mainstem and tributary sites. We compare results from eDNA surveys with other available data (from spawner surveys, radiotelemetry, and smolt traps) and species-specific observations made in the field and make recommendations on using this tool to document recolonization patterns of migratory fish species.

2 | STUDY AREA

The Elwha River is located 10 km west of the city of Port Angeles (Washington State, USA) and flows northward for 72 km from headwaters originating in snowfields within the heart of the Olympic Mountains (Figure 1). Geomorphically, the Elwha River occurs as a series of alternating floodplain reaches separated by canyon reaches. The Lower Elwha (LE) is the reach downstream of the Elwha dam, the Middle Elwha (ME) occurs between Elwha Dam and Glines Canyon Dam, and the upper Elwha (UE) consists of the Mills, Geyser Valley, Elkhorn, Hayes, and Wilder floodplain reaches, each separated from the other by a canyon, including the 5-rkm-long Grand Canyon of the Elwha. The mainstem river is fed by 20 named tributaries (Brenkman et al., 2008) and many 1st- and 2nd-order unnamed tributaries. Within Olympic National Park, the river flows through old-growth coniferous forests in the upland and mixed deciduous riparian areas dominated by

black cottonwood (*Populus balsamifera* ssp.), red alder (*Alnus rubra*), and western red cedar (*Thuja plicata*). Once the river leaves the National Park, it travels through mixed land ownerships (private, state, and tribal), second-growth forest, and increased human development until it enters the Strait of Juan de Fuca, a waterbody connecting Puget Sound and the Strait of Georgia to the Pacific Ocean. The 833 km^2 watershed is partially in the rain shadow of Mt. Olympus and the Bailey Range, creating an annual average precipitation gradient ranging from 550 cm in the headwaters to 100 cm near the mouth of the river. The hydrograph averages 43 cubic meters per second (cms) and is bimodal, a condition created by wet winters (both precipitation and snow) and snowmelt in the spring (Duda, Warrick, & Magirl, 2011).

Prior to construction of the Elwha Dam in 1912, the Elwha River was a highly productive salmon river with five species of Pacific salmon (*Oncorhynchus* spp.; Pink, Chum, Coho, Chinook, Sockeye) and Steelhead Trout occurring in the watershed (Pess et al., 2008; Wunderlich et al., 1994). Additionally, other anadromous species including Pacific Lamprey (*Entosphenus tridentatus*), Coastal Cutthroat Trout (*O. clarkii clarkii*), and Bull Trout (*Salvelinus confluentus*) were present throughout the river (Brenkman et al., 2008). The Elwha Dam was built 7.9 rkm from the mouth of the river without any provision for fish passage, limiting anadromous life histories to the area downstream of the dam. The construction of the Glines Canyon Dam in 1927 at rkm 21.6 further restricted fish movements in the basin and contributed to degrading downstream conditions for fish, particularly due to sequestration of
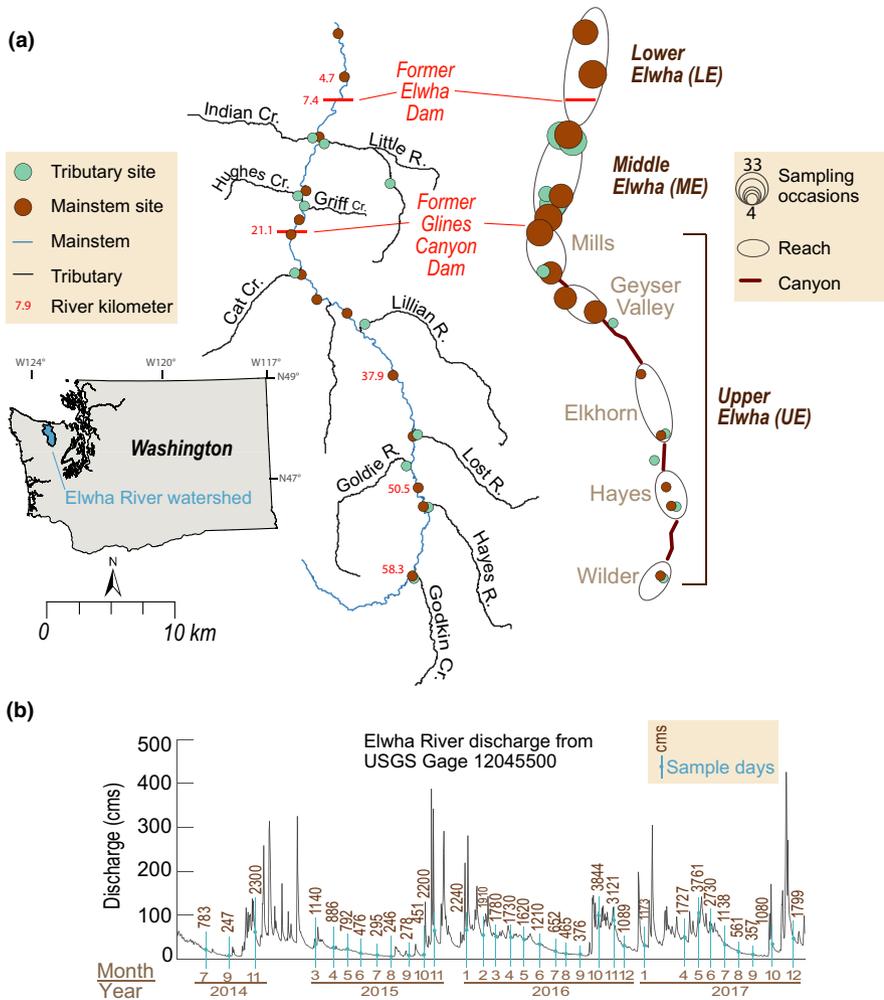
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FIGURE 1 (a) Locator map showing eDNA sampling locations in mainstem and tributary habitats. Former Elwha Dam and Glines Canyon Dam sites indicated, along with floodplain areas referred to in the text, with locations scaled to the number of sampling occasions per site on the Elwha River (see Table 3 for additional information on sample locations). (b) The average daily discharge (cubic meters per second) from continuously recorded measurements at the USGS stream gage 12045500, with the average discharge for the eDNA sampling days indicated on the graph as blue circles

sand, gravels and large woody debris within the reservoirs, increased water temperatures, and loss of spawning and rearing habitat. As seen in other rivers worldwide (Pringle, Freeman, & Freeman, 2000; Reidy-Liermann, Nilsson, Robertson, & Ng, 2012), dam construction and associated disruption of ecosystem processes and function (e.g., hydrological regime, sediment and wood supply, bed armoring) reduced population sizes of Pacific salmon and other migratory fish up to 98% in the Elwha River ecosystem (Pess et al., 2008). Three species of Elwha River fish that we targeted for our eDNA study are currently listed as threatened under the U.S. Endangered Species Act (Chinook Salmon, Steelhead, and Bull Trout).

The decision to remove both dams followed the passage of a 1992 Federal Law calling for the restoration of the Elwha River fisheries and its ecosystem (The Elwha River Fisheries and Ecosystem Restoration Act, PL 102-485; Winter & Crain, 2008). Over 1,500 dams have been removed in the United States since 1973 (American Rivers, 2019) with the median height of these removed dams estimated at about 3 m (Bellmore et al., 2017). Removal of the 64-m-tall Glines Canyon Dam and the 32-m-tall Elwha Dam, coupled with the fact that over 21 million m^3 of sediment was contained within

the reservoirs (Randle, Bountry, Ritchie, & Wille, 2015; Ritchie et al., 2018; Warrick et al., 2015), made the size and scope of the Elwha dam removal project unprecedented. Thus, the decision was made to simultaneously remove both dams over a 3-year period in a staged fashion, gradually removing the dams over an extended period to minimize negative sediment impacts (Magirl et al., 2015; Ritchie et al., 2018) to resident and anadromous fishes (Peters, Liermann, McHenry, Bakke, & Pess, 2017) and their habitat (East et al., 2015; Randle et al., 2015), as well as municipal and industrial water supplies.

Prior to dam removal, all anadromous fish were presumed to occur downstream of the Elwha Dam, except for Sockeye Salmon which were extirpated following dam construction. However, Sockeye Salmon were known to occasionally stray from other rivers into the Elwha. Potamodromous native fish, including Rainbow Trout (the resident life-history form of *O. mykiss*) and Bull Trout, occur throughout the Elwha River from headwaters to mouth and in most tributaries. Eastern Brook Trout (*S. fontinalis*) are a non-native species that were stocked in the Elwha watershed until 1976 and inhabited some floodplain areas and tributaries between the two dams (Brenkman et al., 2008). Brook Trout were observed

downstream of the Elwha Dam on one occasion in a smolt trap but were not detected in electrofishing surveys in the mainstem Lower Elwha (downstream of Elwha Dam) prior to dam removal (Brenkman et al., 2008; Connolly & Brenkman, 2008). Resident and anadromous forms of Coastal Cutthroat Trout were present in the Elwha River prior to dam removal and thought to be present in Indian Creek and Little River (Figure 1) based on limited empirical data (Brenkman et al., 2008). Kokanee Salmon (landlocked Sockeye Salmon) occur in Lake Sutherland, the headwaters of Indian Creek, which is a tributary between the two dams. Genetic analyses of this population suggest that they are distinct from both the most commonly planted Kokanee Salmon stock in western Washington and a nearby population in Lake Ozette (Winans et al., 2008). The status of lamprey throughout the Elwha was unknown, but Pacific Lamprey were rare and limited to the mainstem river downstream of Elwha Dam prior to dam removal.

3 | METHODS

3.1 | Species-specific assay design

Our objective was to design eight species-specific PCR assays for targeted fish species that could potentially recolonize the Elwha River watershed (Table 1). We also developed assays for Pacific Lamprey/*Lampetra* spp. and Sockeye Salmon, for which assay development is described in Ostberg, Chase, Hayes, and Duda (2018) and Tillotson et al. (2018), respectively. We obtained archived fin tissue samples for each target species from the Elwha and other neighboring rivers from collections at Washington Department of Fish and Wildlife, Olympic National Park, National Oceanic and Atmospheric Administration, and U.S. Geological Survey (USGS).

We used DNeasy Blood and Tissue Kits (Qiagen, Valencia, California) to extract DNA from tissues. From the tissue samples,

TABLE 2 Assay names and sequence information for forward primer, reverse primer, and probe for studies of fish recolonization of the Elwha River

Species	Assay name (gene)	Size (bp)	Forward primer (5′ – 3′) Reverse primer (5′ – 3′) Probe (5′ – 3′)
Chinook Salmon	CKCO3_464-534 (CO3)	71	ATTCCATGGCCTACACGTGATT GGTATTGGACCTGTCGCAGAAG 6FAM-ATCAACCTTTCTAGCCGTT-MGB-NFQ
Chum Salmon	CMCO3_812-893 (ND3)	73	CTCCCTATGAGTGCGGATTTG GCAATTAAAAAGAAGCGTAAAGAGAAG 6FAM-CCCACTAGGGTCCGC-MGB-NFQ
Coho Salmon	COCytb_980-1093 (Cyt B)	114	CCTTGGTGGCGGATATACTTATCTTA GAACTAGGAAGATGGCGAAGTAGATC 6FAM-TGGAACACCCATTCAT-MGB-NFQ
Pink Salmon	PKCytb_906-1000 (Cyt B)	95	GGTTGTCCCCATTCTACACACAT TGAGTATATCCGCTACTAAAGCTCAAAA 6FAM-ACGAGGATTAACCTTTCGACCAC-MGB-NFQ
Sockeye Salmon	SECO3_861-930 (ND3)	70	TCTGCCCTTCTCCTTACGATTTT AAGGGCGATTTCTAGGTCGAAC 6FAM-CCATCCTGTTCCTCCT-MGB-NFQ
Rainbow Trout/Steelhead	RTCytb_793-878 (Cyt B)	86	CCACCTCATATTAAACCCGAATGA GCAAGTACTCCTCCCAGCTTGTT 6FAM-AATCCTACGATCCATCCC-MGB-NFQ
Cutthroat Trout	CCCytb_572-685 (Cyt B)	114	CCGCTACAGTCCTTCACCTTCTA GATCTTTGTATGAGAAGTAAGGATGGAA 6FAM-TGAGACAGGATCCAAC-MGB-NFQ
Bull Trout	BLCytb_771-854 (Cyt B)	84	ACCAGCCAACCCCCTAGTTA GGGATAGATCGGAGAATTGCAT 6FAM-ACCACACATCAAGCC-MGB-NFQ
Brook Trout	BKCO3_314-388 (CO3)	75	AGAAGGAGAGCGGAAACAAACC TGAAGGAAAGTAAAATAGAACCCCAAT 6FAM-TCCAAGCTCTTACTCTCACC-MGB-NFQ
Pacific Lamprey	ETCytb_890-1015	126	CTTTAGCAGCAGCCATCATA GTAGTGCTAGATCAGCGATTAGAA 6FAM-TAT + CCAATT+CCG + CCC+AC-ZEN-Iowa Black FQ[a]
River Lamprey	LACytb_890-1015	126	CTTTAGCAGCAGCCATCATA GTAGTGCTAGATCAGCAATTAGAA 6FAM-CAT + TCAATT+TCG + TCC+GC-ZEN-Iowa Black FQ[a]

[a]+N locked nucleic acid.

TABLE 3 Locations (latitude and longitude) and sampling schedule (months of year) of eDNA sampling in the Elwha River (type = mainstem [MS]) and its tributaries (TR)

Site	Sect.	Type	Lat	Lon	Rkm	Occn	Annual monthly sampling dates			
							2014	2015	2016	2017
Elwha Road	LE	MS	48.144216	−123.562141	0.6	27	8,9,11	3,6,8	1–12	1,4–10,12
Bike Bridge	LE	MS	48.113771	−123.553748	4.7	33	8,9,11	3–11	1–12	1,4–10,12
Aldwell Reservoir	ME	MS	48.068552	−123.578635	11.7	33	8,9,11	3–11	1–12	1,4–10,12
Indian Creek	ME	TR	48.066641	−123.584753	12.0	33	8,9,11	3–11	1–12	1,4–10,12
Little River	ME	TR	48.064081	−123.576876	12.2	33	8,9,11	3–11	1–12	1,4–10,12
Little River above barrier	ME	TR	48.039374	−123.501428	12.2	12	8,9,11	3,6,8	1–4,9	9
Boulder Garden	ME	MS	48.031755	−123.592504	16.6	30	8,9,11	3,4,6,8–10	1–12	1,4–10,12
Hughes Creek	ME	TR	48.025313	−123.596892	17.6	10	8,9,11	3,6,8	1,3,9	9
Griff Creek	ME	TR	48.016079	−123.592217	17.8	27	8,9,11	3,6,8	1–12	1,4–10,12
Altair Bridge	ME	MS	48.010943	−123.590127	19.5	32	8,9,11	3–11	1–12	1,4–10,12
Glines	UE	MS	48.000424	−123.601121	21.3	31	9,11	3–11	1–12	1,4–10
Whiskey Bend	UE	MS	47.972863	−123.591391	24.8	21	9	3, 5–8	3–10	1,4,5,7–10
Cat Creek	UE	TR	47.972902	−123.592966	24.8	7	9	6,8	9,12	8,9
Goblin Gates	UE	MS	47.955523	−123.573564	27.6	23	9	3,6,8	1–11	1,4–10
Dodger Point Bridge	UE	MS	47.533353	−123.322908	31.1	23	9	3,6,8	1–11	1,4–10
Lillian River	UE	TR	47.937714	−123.521275	32.9	4	9	8	9	9
Mary's Falls	UE	MS	47.904377	−123.491040	37.9	4	9	8	9	9
Elkhorn	UE	MS	47.861163	−123.468357	43.2	4	9	8	9	9
Lost River	UE	TR	47.861519	−123.468133	43.2	4	9	8	9	9
Goldie River	UE	TR	47.839760	−123.472348	46.7	3	na	8	9	9
Chateau Camp	UE	MS	47.814561	−123.454941	50.5	3	9	na	9	9
Hayes River	UE	TR	47.807606	−123.452795	51.4	4	9	8	9	9
Hayes Floodplain	UE	MS	47.807564	−123.453266	51.4	4	9	8	9	9
Godkin Creek	UE	TR	47.758986	−123.463761	58.3	4	9	8	9	9
Wilder Floodplain	UE	MS	47.759058	−123.465240	58.3	4	9	8	9	9

Note: Tributary sampling generally occurred within 100 m of the confluence with the mainstem. When mainstem and tributary pairs occur, mainstem sampling always occurred upstream of the tributary confluence (Figure 1). River kilometer (rkm) determined using a longitudinal profile of the Elwha River mainstem from the USGS National Hydrography Dataset and the rkm value for tributaries is given for the confluence with the mainstem.

we targeted three mitochondrial DNA (mtDNA) genes, cytochrome b (Cyt*b*), cytochrome oxidase 3 (CO3), and NADH dehydrogenase 3 (ND3), for identification of nucleotides that differentiated the target species. Several representative Cytb, CO3, and ND3 sequences from each target species were retrieved from GenBank and aligned using MEGA 7.0.21 (Kumar, Stecher, & Tamura, 2016). Next, we identified nucleotide regions that were conserved across species for construction of sequencing primers, which were used to amplify and sequence five DNA samples from each species in our target species collection. We sequenced 1,123 bp of Cytb using forward primer Cytb_F1 5′-AAAACCACCGTTGTTATTCAA-3′ and reverse primer Cytb_R1 5′-CCGACTTCCGGATTACAAGA-3′ and deposited sequences from representative samples in GenBank (KU872710–KU872718). We sequenced 1,000-bp covering CO3 and ND3 using forward primer CO3_F2 5′- TCAGGCACTGCAGTCTGATT-3′ and

reverse primers tRNA_Arg_R1 5′-CATAAGGCGGTCATGGACTT-3′ and tRNA_Arg_R2 5′-CTTTTGAGCCGAAATCAAGG-3′ and deposited sequences from representative samples in GenBank (KU872719-KU872727). Sequences were edited and aligned using SEQUENCHER v.4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan).

To develop species-specific assays, we used the sequence data from the target species collection and identified regions conserved within species where nucleotide mismatches were maximized among the nontarget species. Because many of our target species were closely related (e.g., Chinook Salmon/Coho Salmon and Chum Salmon/Pink Salmon), maximizing the number of mismatches in the primer/probe design minimized amplification of nontarget species eDNA (and, thus, false-positive detections). Further, it would not be unusual for any given eDNA sample from

the Elwha watershed to consist of a mixture of multiple targeted species. Therefore, when possible, we used candidate primer sites with mismatches near the 3' end to increase assay specificity (Wilcox et al., 2013).

Real-time PCR assays were designed for Chinook Salmon, Coho Salmon, Pink Salmon, Chum Salmon, Steelhead/Rainbow Trout, Coastal Cutthroat Trout, Bull Trout, and Brook Trout in the Elwha River and near vicinity using Primer Express 3.0.1 (Applied Biosystems, Foster City, California) (Table 2). Each species-specific primer/probe design was tested in silico for specificity against all co-occurring fish species in the Elwha using GenBank Primer-BLAST and BLAST; no highly homologous matches were returned (see Table S1 for primer/probe nucleotide mismatches in Elwha salmonids). Each probe was designed as a minor groove binding probe with a 5' FAM label and 3' nonfluorescent quencher.

In vitro testing of each assay was performed on genomic DNA from target and nontarget species. All PCR assays contained 1× Gene Expression Mastermix (ThermoFisher Scientific, Waltham, Maine), 1× custom TaqMan primerand probe mix (a final concentration of 450 nM for each forward and reverse primers and 125 nM probe) and were run on a ViiA7 Real-Time PCR system (Applied Biosystems) using the following default cycle parameters, unless indicated otherwise: initial steps of 2 min at 50°C then 10 min at 95°C, followed by 45 cycles of denaturing at 95°C for 15 s, and annealing/extension at 60°C for 1 min. Results were analyzed using ViiA 7 RUO 1.2.4 software. Specificity tests revealed that each assay only amplified in the target species, with the exception of the Chinook Salmon assay (CKCO3_464-534) which also amplified in 100 pg Chum Salmon and the Pink Salmon assay which also amplified in 100 pg Chinook Salmon, Sockeye Salmon, and Coastal Cutthroat Trout (Table S2). Neither of these assays amplified in 10 pg nontarget species. We believe these assays are functional despite cross-amplification at a high concentration of genomic DNA because a 1-L aquatic eDNA sample is not likely to yield 100 pg DNA from single species. In addition, the Coastal Cutthroat Trout assay was also tested against Westslope Cutthroat Trout (*O. c. lewisi*) and Yellowstone Cutthroat Trout (*O. c. bouvieri*). These non-native cutthroat trout could possibly be present due to past introductions in Olympic National Park, but results indicated that nontarget cutthroat trout did not amplify.

We determined the limit of detection (LOD), limit of quantification (LOQ), and efficiency of each assay (Table S3). This was accomplished by designing gBlock double-stranded gene fragments (Integrated DNA Technologies, Coralville, Iowa) representing the species amplicon for each assay and performing real-time PCR on a dilution series consisting of 10,000, 1,000, 100, 10, 5, and 1 copies per reaction with 40 replicate samples at each concentration, following the reaction chemistry and cycling parameters described above. The curve-fitting modeling method described in Klymus et al. (2019) was used to estimate LOD and LOQ. A 95% probability of detection criteria was applied for LOD estimates, and a 35% coefficient of variation criteria was applied for LOQ precision.

3.2 | Field sampling

We established a network of 25 eDNA field sampling locations throughout 56 rkm of the mainstem Elwha River (*n* = 14 sites) and in 10 major tributaries (*n* = 11 sites) (Figure 1a). From 2014 to 2017, sampling occurred downstream of Elwha Dam (LE; *n* = 2 sites) and in-between the dams (ME; *n* = 8 sites) in areas accessible by road. All sites upstream of the Glines Canyon Dam (UE; *n* = 15 sites) were in roadless backcountry, with the sites in Mills and Geyser Valley floodplains requiring a full day hike for access and sampling. Cat Creek was also accessible during a day trip but required a crossing of the mainstem of the river and for safety reasons was inaccessible during some times of the year with higher flows. The uppermost sites, in Elkhorn, Hayes, and Wilder floodplains (i.e., sites from rkm 37.9 to rkm 58.3) required overnight backpacking trips lasting a minimum of 5 days to complete sampling. All UE floodplain sections had sampling locations near the upstream and downstream end, except the uppermost Wilder floodplain, which had a single mainstem sampling location. We avoided placing mainstem Elwha River sites immediately downstream of tributary junctions. Thus, for sites near roads or accessible by foot in a day, we collected eDNA samples approximately monthly for the duration of the study (with some exceptions noted below). For sites that required multiday backpacking trips, we conducted sampling during summer base flows (in either August or September) (Table 3). The number of sampling occasions at each site ranged from 3 to 33 (Table 3).

We placed sampling sites on every major tributary that was expected to potentially harbor anadromous salmon following dam removal (Brenkman et al., 2008). For tributaries, we typically sampled <100 m upstream from the confluence with the Elwha River. Little and Lillian Rivers each had one site upstream of barrier falls (e.g., inaccessible to salmon). Those two sites served as negative field controls for all targeted species except Bull Trout, Rainbow Trout, and Cutthroat Trout.

Resource limitations during early project sampling in 2014 and the winter of 2015 precluded monthly sampling, which began in March of 2015 and continued until December 2017. Weather events and logistical issues precluded sampling during four of these time periods in December 2015 and February, March, and November of 2017 (Table 3). Streamflow conditions varied across all sampling events. We used data from a USGS streamflow gage (12045500, USGS, 2019) located between the former dam locations at rkm 13.5 to calculate an average daily flow for the sampling events that occurred during each sampling trip. Average daily streamflow for sampling events ranged from 7 to 109 cms, with the lowest flows occurring during the summer months (July–September) and the highest flows occurring in the winter and spring (Figure 1b).

To survey eDNA, we collected three replicate 1-L subsurface water samples at each site and filtered the water through a pre-sterilized 47-mm-diameter Nalgene filter funnel with a 0.45-μm pore size cellulose nitrate sterile filter membrane (GE Healthcare, Marlborough, MA). The filter funnel was connected to a Masterflex

L/S Easy-Load peristaltic pump powered by a 12 v cordless screw-driver. After filtration, filters were removed from the funnel and placed in sterile 5-ml tubes containing 95% ethanol. Upon return to the laboratory, samples were stored at −20°C until DNA extraction within 48 or 96 hr for front-country and backcountry samples, respectively. Negative controls consisted of a 1-L bottle of store-bought purified water that was filtered in the field. At front-country sites, two negative control samples were collected each day of sampling; one in the middle of the sampling session and the other at the end. For backcountry sites, a negative control sample was collected at each site as the last sample processed. For each water sampling event, individually bagged sampling kits containing a funnel loaded with a filter, unused 475-ml plastic cup, forceps, applicator stick (to help fold the filter), gloves, and 5-ml tube with ethanol were prepared prior to field sampling and care was taken to avoid cross-sample contamination in the field. We sterilized all filter funnels and forceps prior to use by soaking in 10% bleach for at least 10 min followed by rinsing with filtered water.

3.3 | Laboratory analyses

All laboratory protocols and analyses were designed to avoid cross-contamination (Goldberg et al., 2016). The eDNA workflow and sample preparation were separated into designated work rooms including a clean room where DNA was extracted (no amplified PCR products or highly concentrated target DNA sequences allowed), a second room where PCR reagents were prepared and loaded, a third room where DNA standards were diluted and loaded, and a fourth room dedicated to PCR amplification. Sample preparation was performed in UV hoods using equipment dedicated to processing eDNA samples at each workstation. Workstations were decontaminated with UV and/or 10% bleach before and after each use.

At the start of the study, we used one half of each filter for DNA extraction and the remaining half was archived. This was to ensure that we could re-analyze samples in the event of contamination, particularly important for backcountry sites that were only sampled once each year. Each filter half used for DNA extraction was cut into 2-mm strips with a sterile scissor and incubated in Lysis buffer for one hour at 55°C prior to DNA extraction. Starting in July of 2016, confident that our procedures were not causing any contamination, we no longer archived and used the entire filter for extractions. DNA was extracted using DNeasy Blood and Tissue kits (Qiagen) with the following modifications: 360 µl ATL buffer and 40 µl of Proteinase K were used for cell lysis and the volume of AL buffer and 100% ethanol was adjusted to 400 µl postlysis. DNA was eluted in 200 µl AE buffer and stored at −20°C until PCR analysis.

All DNA extracted from water samples were tested first for the presence of PCR inhibitors by performing an internal positive control (IPC) assay using TaqMan Exogenous Internal Positive Control Reagents (EXO-IPC) (Applied Biosystems). Each DNA sample was run in duplicate, and each IPC assay was performed in 10 µl volumes consisting of 5 µl of Gene Expression Master Mix, 1 µl EXO-IPC mix,

0.2 µl EXO-IPC DNA, 0.8 µl Nanopure sterile H_2O, and 3 µl DNA template or sterile water for the nontemplate control (NTC) using the default cycle parameters with 40 cycles. Environmental samples were considered inhibited when samples displayed a >3 cycle threshold (C_t) shift relative to the mean NTC. Samples that were inhibited were treated with OneStep PCR Inhibitor Removal Kit (Zymo Research Corporation, Irvine, California) and retested with the IPC assay to confirm that PCR inhibition was alleviated.

We used the eight salmonid eDNA assays developed herein along with a Sockeye Salmon assay (Tillotson et al., 2018), Pacific Lamprey assay, and *Lampetra* spp. assay (Ostberg et al., 2018) to interrogate DNA extracted from the Elwha River water samples. All species-specific assays were performed in triplicate on each Elwha River sample, resulting in a total of 9 PCR replicates per site-sample event. For the Lamprey assays, we used 14 µl volumes following the reaction chemistry and cycling parameters described in Ostberg et al. (2018). For all other assays, we used 10 µl reaction volumes for ½ filters and 12 µl reaction volumes for full filters, following the reaction chemistry and cycling parameters described above, with the exception that in 12 µl reaction volumes we used 375 nM and 105 nM final concentrations for the primers and probe, respectively. A six-point standard curve consisting of a serial dilution of 10–100,000 copies per reaction was run in quadruplicate for each species assay. Field negative controls, extraction negative controls, and no-template controls (sterile water in place of DNA) were included on each real-time PCR plate. We considered a positive detection as any sample amplifying at less than 40 Ct with a uniform curve morphology, as suggested by Klymus et al. (2019). In summarizing the annual detection history for each site-sample occasion, we differentiated "trace" detections as those cases where only 1 of 9 sample-site replicates were positive from those cases where ≥2 replicates were positive. Thus, for a given taxa, if the only detection at a site across a whole year were one or more cases with 1 of 9 replicates being positive, these were labeled as trace detections for the year.

3.4 | Multiscale occupancy modeling

To analyze the data collected during surveys of salmonid and Pacific Lamprey eDNA, we fit multiscale occupancy models using the R package eDNAoccupancy (Dorazio & Erickson, 2018). For each migratory salmon species, the parameters of a multiscale occupancy model were estimated for the adult immigration and spawning season in each of the four study years. Each species-specific adult immigration and spawning season was derived from long-term monitoring records in the Elwha (defined in Figure S1). The temporal extent of a season differed among species according to its migratory behavior and life history. Migration timing for all migratory salmonid species in the Elwha was compiled or inferred from ongoing data collection efforts, including SONAR, redd surveys, and smolt trapping. This resulted in estimates for three life cycle periods specific to the Elwha River system: (1) adult immigration and spawning; (2) age 0 + smolt rearing and outmigration; and (3) age 1 + smolt rearing and outmigration (Figure S1). For purposes of multiscale occupancy modeling,
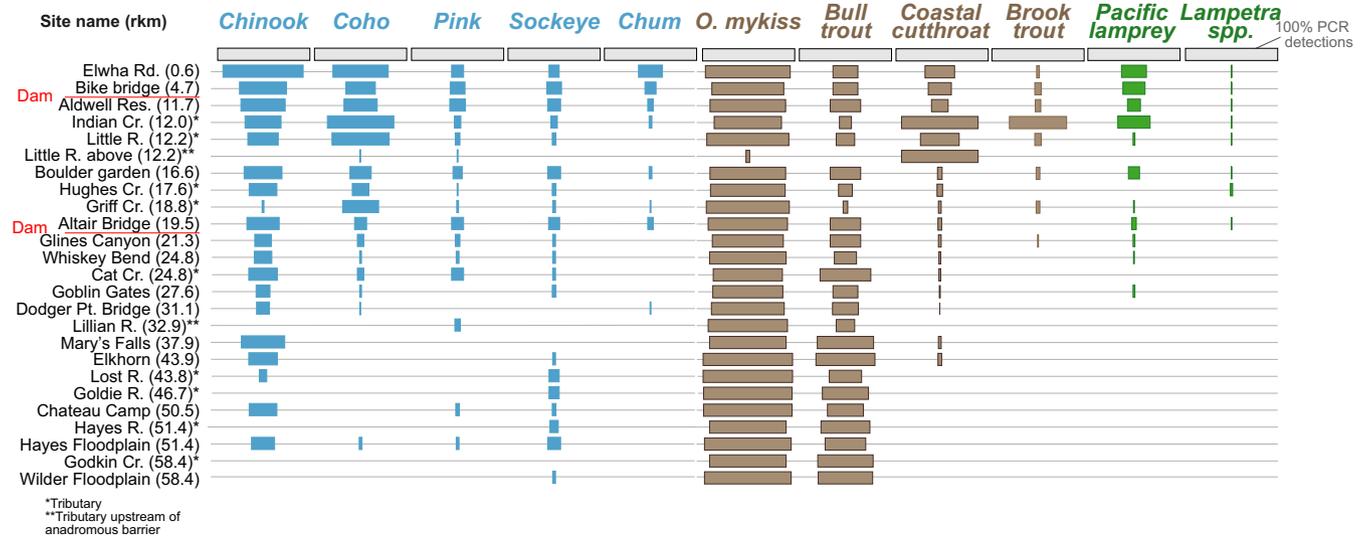
FIGURE 2 Funnel plot representation of real-time PCR detections as the percentage of total PCRs from all sample occasions. Values specific to each taxa and location along a longitudinal profile of the Elwha River. Gray bars indicate 100% possible detections, each site's distance from the river mouth given in rkm in parenthesis (for tributaries this value is for the confluence with the Elwha River mainstem)

we only used eDNA water samples from the adult immigration and spawning period. Over the course of the study, there were four available seasons for each targeted migratory species, one from each year of the study. For Brook Trout, Rainbow Trout, and Bull Trout, we used all sampling events from a calendar year.

Quantiles of the posterior distribution of model parameters were estimated using ergodic averages of a Markov chain of length 100,000 after discarding the first 10,000 elements of the chain to exclude transient behavior. This reduced the Monte Carlo error of the parameter estimates to allow reproducible reporting of results.

In the occupancy model, the site-level probability of occurrence of eDNA (parameterized by ψ) was specified as a function of the distance upriver (rkm). The conditional probability of occurrence of eDNA in a sample of an occupied site (parameterized by θ) was assumed to be constant during each year. Similarly, the conditional probability of detection of eDNA in a PCR replicate of a sample that contained eDNA (parameterized by p) was assumed to be constant during each year. Relatively simplistic models of θ and p were assumed because potential sources of variability in these parameters were not measured.

4 | RESULTS

Our sampling from August 2014 to December 2017 yielded eDNA water samples from a range of 3 to 33 sampling occasions at our 15 front-country and 10 backcountry sites (Figure 1a). This sampling design resulted in 412 site-sample events, with field ($n = 3$) and technical replicates ($n = 3$) for 11 species-specific assays yielding 39,419 PCRs (not including negative and internal controls; Duda et al., 2020). The total number of positive PCR detections—7,492 (19.0%)—was unevenly distributed among sample locations and species (Figure 2). A total of 558 (1.4%) PCRs did not meet the 40 C_t threshold (average

$C_t = 41.6$) recommended by Klymus et al. (2019) and were scored as nondetections. These cases occurred in each assay and across multiple sites and sampling occasions, with the most found in Coho Salmon (164) and Pacific Lamprey (153) and the fewest in Pink Salmon (9), Sockeye Salmon (8), and *Lampetra* spp. (2). No internal or laboratory-based negative controls yielded positive detections.

4.1 | Pacific Salmon

Prior to dam removal, anadromous Pacific salmon were restricted to areas downstream of the Elwha Dam, aside from some smolts produced by resident Rainbow Trout upstream of the dams (Hiss & Wunderlich, 1994). Surveys of eDNA showed different spatial and temporal patterns for all five Pacific salmon species (Figures 2 and 3). After dam removal, eDNA from all targeted anadromous salmonids was detected upstream of the former Elwha Dam location in every year studied, except for Pink Salmon and Chum Salmon nondetections in 2016 (Figure 3). The four tributaries of ME were all occupied in at least 1 year by all species of Pacific salmon, except for Chum Salmon, which was never detected in Hughes Creek or the Little River (Figure 3).

All salmon species were detected upstream of the former Glines Canyon Dam (Figures 2 and 3). Chinook Salmon were detected in most UE floodplains, including in the former Lake Mills floodplain (sites spanning rkm 21.3 to 24.8; all four years); Geyser Valley, (rkm 27.6 to 31.1; in 3 of 4 years); Elkhorn (rkm 37.9 to 43.2; in 2 of 4 years), including a positive detection three weeks after the final blast of dam removal that allowed fish passage); and in Hayes floodplain (rkm 51.4) in a single year. Chinook Salmon were not detected at Wilder (rkm 58.3), the uppermost floodplain (Figure 3).

Coho Salmon eDNA was detected upstream of the former Glines Canyon Dam in the former Lake Mills and Geyser Valley
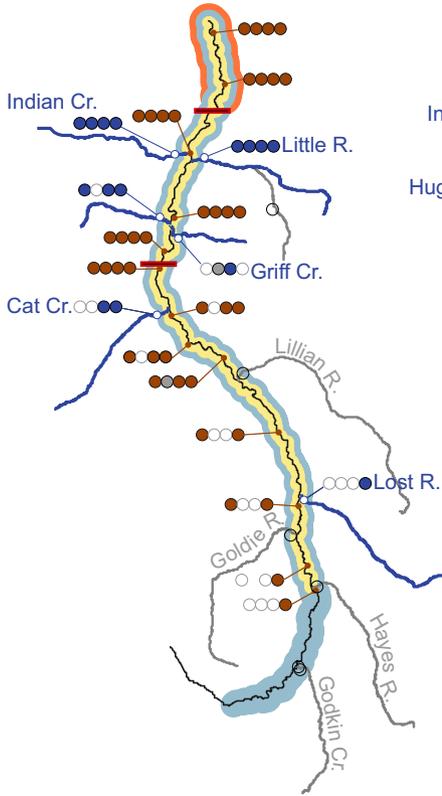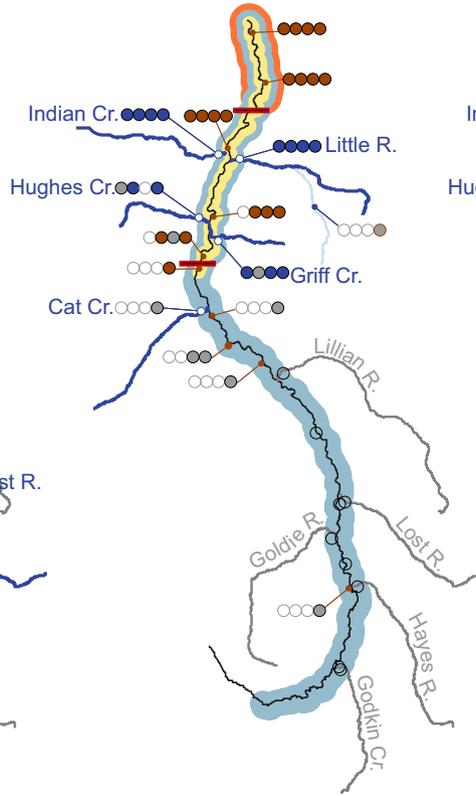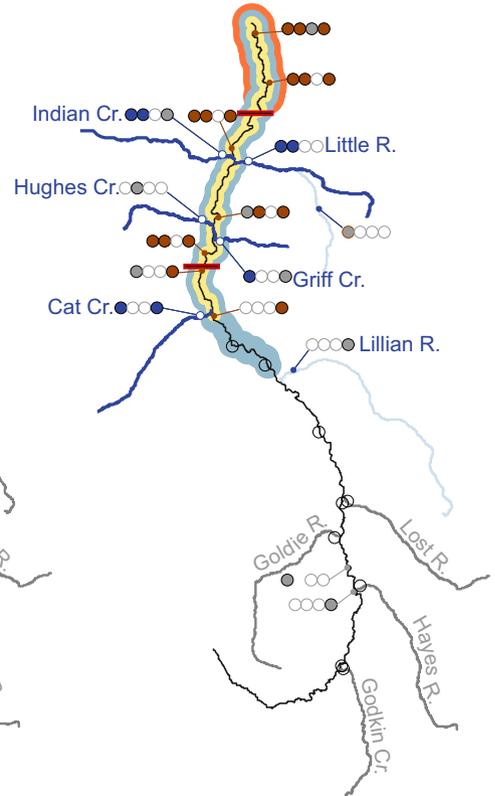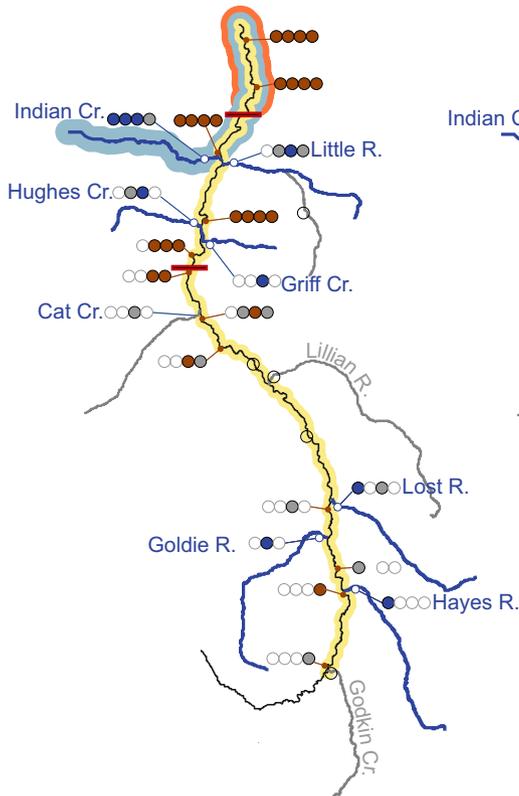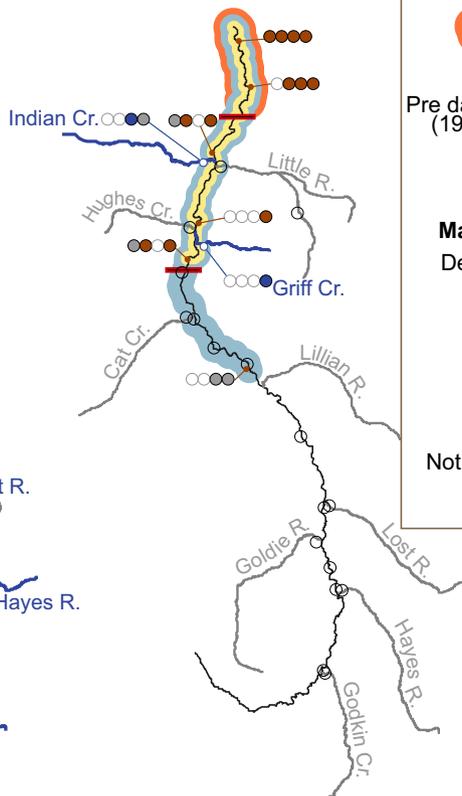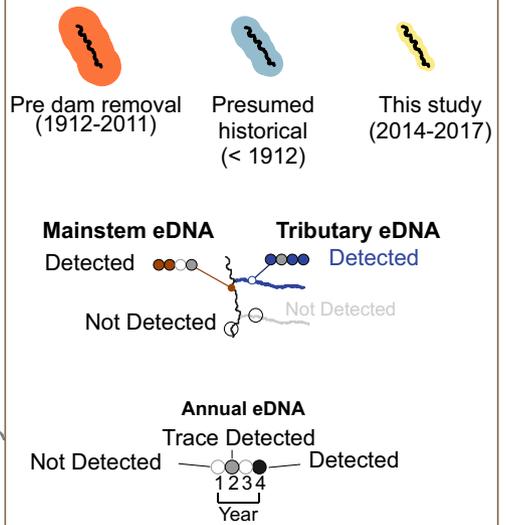
FIGURE 3 Annual detection summary for migratory salmonids in the Elwha River based on eDNA sampling. For each mainstem and tributary sampling location, open black circles represent cases where the taxa were not detected. When detected at a site, the detection history is given for each year of the study (typically four). The possible states for each year are no detection (gray open circle), detection (brown or blue filled circle for mainstem and tributary, respectively), or trace detection (filled dark gray; representing those cases with detections of a single PCRs of nine possible being positive). Highlighting of the Elwha River mainstem indicates the taxon's before dam removal distribution (orange), after dam removal distribution (yellow), and presumed distribution prior to the dams being built (blue)

floodplains in 2017. Coho Salmon were also detected in Geyser Valley in 2016 and Hayes in 2017, but only at a trace level (i.e., the 1 of 9 positive detections; Figure 3). Pink Salmon eDNA was detected in the former Lake Mills floodplain in 2017, had trace detections at Hayes floodplain in 2014 and 2017, and was undetected in other upstream floodplains (Figure 3). Sockeye Salmon eDNA was detected in Lake Mills floodplain in 3 of 4 years, in addition to samples in all the other floodplains further upstream. The trace detection of Sockeye Salmon eDNA at Wilder in 2017 represented the uppermost detection of any Pacific Salmon. Finally, Chum Salmon were only detected in the Geyser Valley floodplain with trace detections in 2016 and 2017.

Cat Creek (confluence with the Elwha at rkm 24.8) was the only tributary sampled between the former Glines Canyon Dam and the Grand Canyon of the Elwha. Chinook, Coho, Pink, and Sockeye were detected in Cat Creek (Figure 3), with Chinook Salmon having higher frequencies of detection than the other three species (Figure 2). In tributaries upstream of the Grand Canyon of the Elwha, only Chinook Salmon (Lost River, 2017) and Sockeye Salmon (Lost and Hayes rivers in 2014 and the Goldie River in 2016) were detected (Figure 3).

Results from the multiscale occupancy modeling of sampling occasions during the time period of each species adult immigration and spawning period were aligned closely with spatial and temporal eDNA results that included all sampling occasions (Figure 6). In the first three years, Chinook Salmon eDNA from samples collected during the adult immigration and spawning season was more prevalent in downstream locations than in areas upstream of the former Glines Canyon Dam. However, by year 4, probability of Chinook Salmon eDNA occurrence (ψ) increased upstream, indicative of recolonization of the upper reaches of the Elwha River. Across all years, θ ranged from 0.58 to 0.96 and p ranged from 0.57 to 0.80. Coho Salmon and Pink Salmon showed a similar pattern as Chinook Salmon, with a higher ψ in downstream reaches in years 1–3 and an increase in upstream in year 4. Across all years, Coho Salmon θ ranged from 0.57 to 0.82 and p ranged from 0.48 to 0.71, while θ ranged from 0.48 to 0.90 and p ranged from 0.28 to 0.75 for Pink Salmon (Figure 6). Sockeye Salmon had modeled ψ that was more uniform in years 1 and 4 and sigmoidal favoring downstream occupancy in years 2 and 3, with lower values for θ (0.45–0.74) and p (0.16–0.46) contributing to higher variance in ψ estimates. In all years, Chum Salmon consistently showed a similar relationship between ψ and distance from the Elwha River mouth, with higher detections downstream of former Glines Canyon Dam and minimal detections upstream and consistently low levels of θ (0.29–0.52) and p (0.12–0.43).

4.2 | Trout and Charr

Spatial and temporal patterns of eDNA detection differed between trout and charr (Figures 2 and 4). Our *O. mykiss* eDNA assay cannot differentiate Rainbow Trout (resident form) from Steelhead (anadromous form), but in this paper, we present the results for *O. mykiss* PCR assays as detections of Rainbow Trout, because they are much more abundant in the watershed than Steelhead (Brenkman et al., 2012). Rainbow Trout eDNA detections were ubiquitous and there were consistently high levels of Rainbow Trout eDNA present across all sampling sites and tributary locations (Figures 2 and 4), with over 50% of the site-sample events being positive in 9 of 9 PCRs. This suggests consistently high levels of eDNA present throughout the Elwha River basin, which would be expected for an abundant species with both resident and migratory (i.e., Steelhead) individuals present throughout the watershed (Brenkman et al., 2008, 2012).

Bull Trout were also ubiquitous, based on detections present at every site and in every sampled tributary except the Little River upstream of a migration barrier (Figures 2 and 4). Bull Trout only showed perfect detection (9 of 9 real-time PCR) in 7% of sampling events. There were 4 times more lower level detections (i.e., 100 instances where 1–3 real-time PCRs were positive from replicate water samples) versus higher level detections (25 instances where 7–9 real-time PCRs were positive) for Bull Trout.

We detected non-native Brook Trout eDNA throughout ME at relatively low levels, except for Indian Creek where we encountered consistently high levels of detection across all survey months and years (Figure 4). Brook Trout eDNA was also detected at low levels in other ME tributaries (Little and Griff) which harbored Brook Trout prior to dam removal (Figure 4). Brook Trout dispersal in UE was limited to two sites, one immediately upstream of the former dam and the other at Whiskey Bend (rkm 24.8). These trace detections occurred at the Glines site on three separate sampling occasions during 2015 to 2017 (Figure 4). Brook Trout eDNA was not detected in any of the sampled tributaries in UE. Multiscale occupancy modeling showed a high probability for Brook Trout moving into areas downstream of the Elwha Dam following dam removal, with low but consistent levels of detection occurring regularly at the two LE sites (Figure 6). The occupancy model also showed a low probability of Brook Trout moving into UE.

We detected Coastal Cutthroat Trout eDNA in all ME tributaries, with consistently high levels of detection in Indian Creek and the Little River (Figures 2 and 4). We also detected Coastal Cutthroat Trout in the ME and LE mainstem, whereas detections upstream of former Glines Canyon Dam were rare and at low levels.
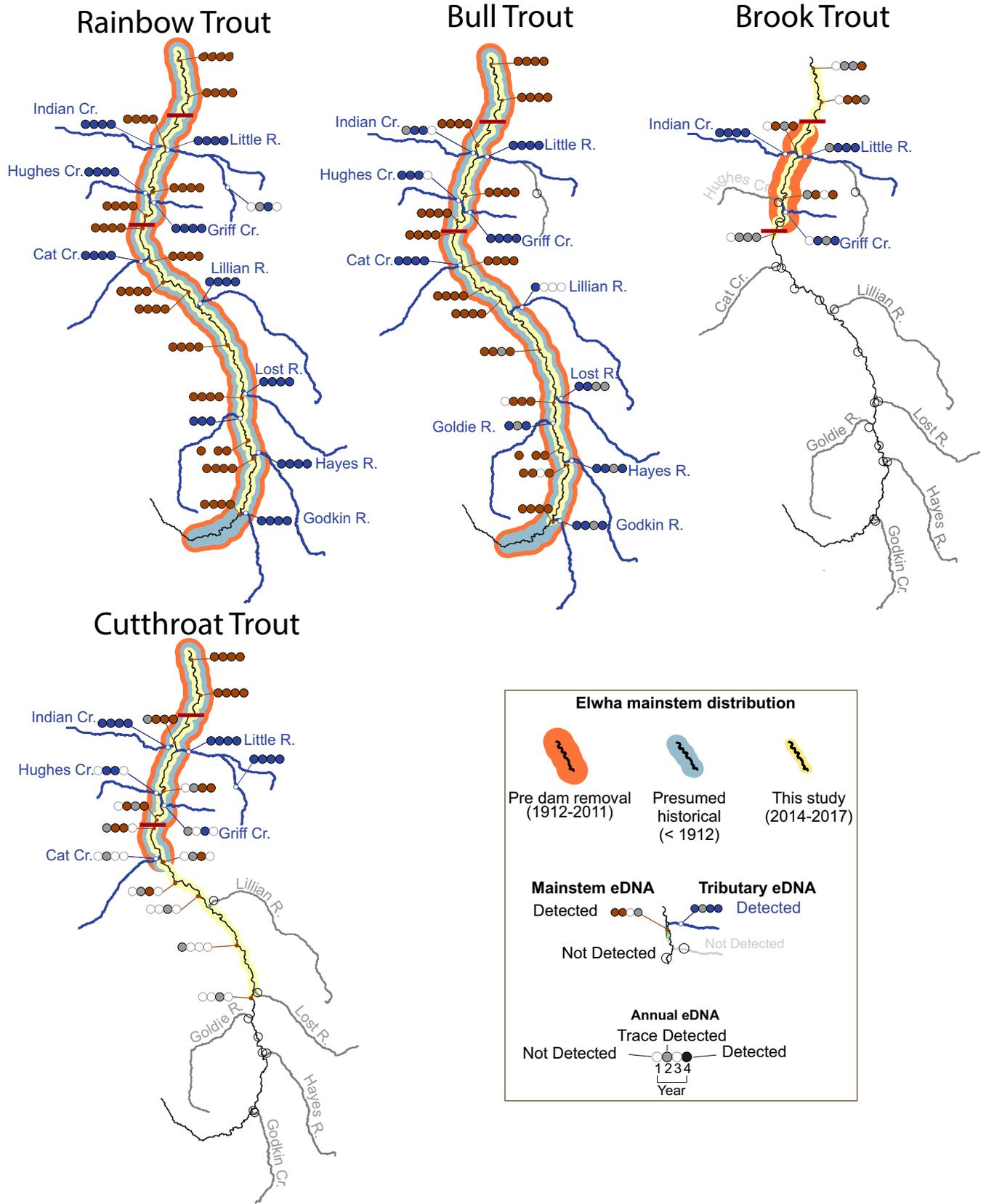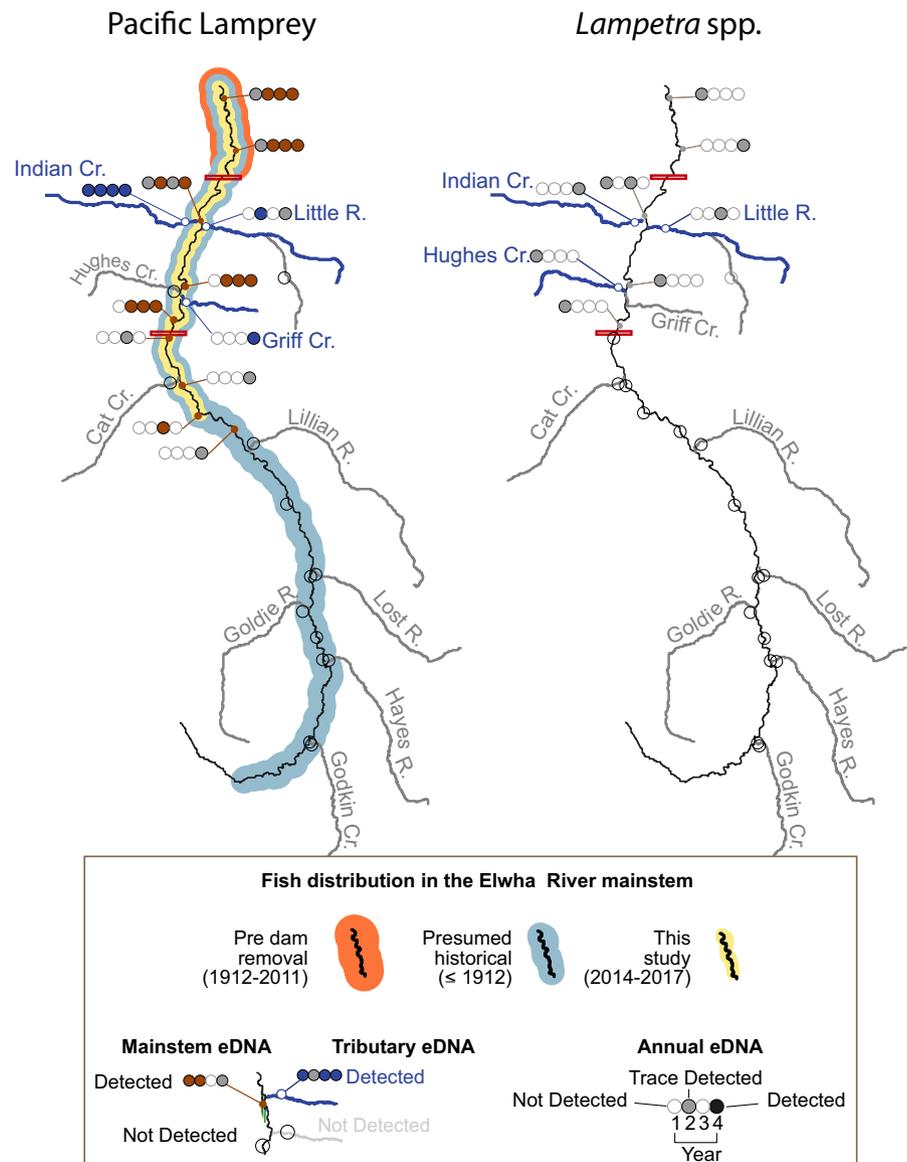
FIGURE 4 Annual detection summary for resident salmonids and charr in the Elwha River based on eDNA sampling. For each mainstem and tributary sampling location, open black circles represent cases where the taxa was not detected. When detected at a site, the detection history is given for each year of the study (typically four). The possible states for each year are no detection (gray open circle), detection (brown or blue filled circle for mainstem and tributary, respectively), or trace detection (filled dark gray; representing those cases with detections of a single PCR reactions of nine possible being positive). Highlighting of the Elwha River mainstem indicates the taxon's before dam removal distribution (orange), after dam removal distribution (yellow), and presumed distribution prior to the dams being built (blue)

FIGURE 5 Annual detection summary for lamprey in the Elwha River based on eDNA sampling. For each mainstem and tributary sampling location, open black circles represent cases where the taxa was not detected. When detected at a site, the detection history is given for each year of the study (typically four). The possible states for each year are no detection (gray open circle), detection (brown or blue filled circle for mainstem and tributary, respectively), or trace detection (filled dark gray, representing those cases with detections of a single PCRs of nine possible being positive). Highlighting of the Elwha River mainstem indicates the taxon's before dam removal distribution (orange), after dam removal distribution (yellow), and presumed distribution prior to the dams being built (blue)

4.3 | Lamprey

Prior to dam removal, Pacific Lamprey were rare and restricted to areas downstream of Elwha Dam. We detected Pacific Lamprey eDNA in the mainstem upstream of both former dam sites, including three tributaries—Indian Creek, Little River, and Griff Creek (Figures 2 and 5). Detections upstream of former Glines Canyon Dam occurred at low levels, both in terms of PCR replicates and the number of sampling occasions. This trend aligned with multiscale occupancy modeling results, which showed a pattern of higher detections of Pacific Lamprey downstream of former Glines Canyon Dam compared to upstream (Figure 6). The range of values modeled for θ (0.27–0.55) and p (0.29–0.45) was similar to values seen in the salmonid species with similar patterns of modeled ψ (e.g., Pink and Chum salmon).

Across all *Lampetra* spp. real-time PCRs, only 0.3% were positive (11/3508). We detected very low levels of *Lampetra* spp. eDNA at 8

sample sites downstream of the former Glines Canyon Dam on five different sampling occasions (Figures 2 and 5). To our knowledge, *Lampetra* spp. (Western River or Western Brook Lamprey) have never been reported in the Elwha watershed.

5 | DISCUSSION

Our study demonstrated that eDNA was an effective tool for documenting the distribution and recolonization of resident and migratory fish species following a large-scale dam removal project on the Elwha River. We collected repeated environmental DNA samples from 2014 to 2017 (in 33 of 40 possible months) at 25 different sites to assess changes in fish species distributions following a large-scale dam removal project. We developed eight species-specific eDNA assays to use with three previously described assays to assess changes in recolonization and spatial extent of fish species following dam
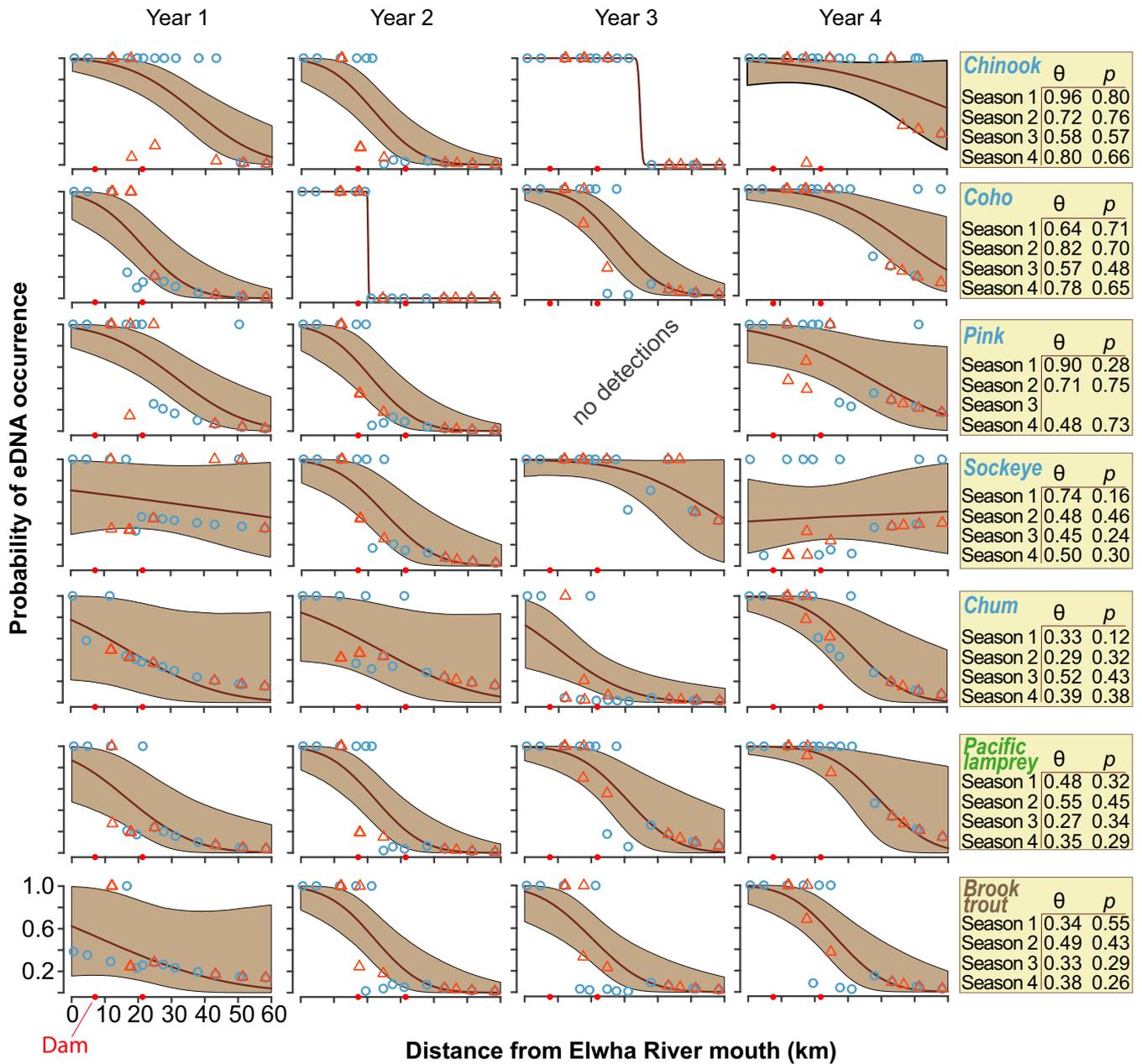
FIGURE 6 Occupancy modeling results showing the estimated relationship (shaded area is the 90% credible interval) between probability of occurrence of migratory fish species and distance upriver for each season of sampling. Seasons were defined as the time period of adult immigration into the system for adult salmon and all calendar year sampling events for Pacific Lamprey and Brook Trout (details in Figure S1). Estimates of occurrence of eDNA at individual sampling locations are overlaid as circles (mainstem Elwha sites) or triangles (tributary sampling locations)

removal on the Elwha River. For anadromous Pacific Salmon and Lamprey, fish had not had access to upstream habitats for nearly a century. Using eDNA was effective for documenting the presence of migratory fish species as they moved into newly accessible habitats upstream of two removed dams. Surveillance of non-native Brook Trout dispersal showed that the species has potentially expanded its range downstream, but not upstream, from areas where it was established between the dams prior to dam removal. When coupled with annual surveys in the difficult to access backcountry of the upper Elwha watershed, eDNA sampling allowed a wide spatial

coverage, spanning 56 rkm of a wilderness river, to determine the longitudinal extent of recolonization. The method also allowed estimation of fish presence in 8 tributaries, where salmon and Pacific Lamprey lacked access for nearly a century. In addition, ongoing field surveys using traditional techniques to document fish recolonization in the Elwha were limited or nonexistent in upper portions of the watershed and some tributaries, especially in the backcountry wilderness, a large portion of the entire basin. Multiscale occupancy modeling (Dorazio & Erickson, 2018) using data from the species-specific adult migration and spawning period (described for each

TABLE 4 Comparison of eDNA results for detections of each migratory species upstream of each dam with results from other ongoing monitoring projects in the watershed

Species	eDNA Detection	1st eDNA observation	eDNA years observed	1st "other method" observation	Other method/source
Chinook	Upstream Elwha Dam	Aug 2014	2014– 2017	Aug 2012	Redd survey[a], Walking survey[c]
	Upstream Glines Canyon Dam	Sep 2014	2014–2017	Sep 2014	Redd survey[a]
Coho	Upstream Elwha Dam	Sep 2014	2014–2017	Oct 2012[d]	Redd survey[c]
	Upstream Glines Canyon Dam	Oct 2016	2016–2017	Oct 2016	Redd survey[c], Radiotelemetry[c]
Sockeye	Upstream Elwha Dam	Aug 2014	2014–2017	Aug 2013	Tangle net[d]
	Upstream Glines Canyon Dam	Sep 2014	2014–2017	Aug 2016	Snorkel survey[c]
Pink	Upstream Elwha Dam	Aug 2014	2014-2015, 2017	Sep 2012	Redd survey[a]
	Upstream Glines Canyon Dam	Aug 2014	2014, 2017	Aug 2019	Snorkel survey[c]
Chum	Upstream Elwha Dam	Aug 2014	2014–2017	Nov 2014	Redd survey[a]
	Upstream Glines Canyon Dam	Jan 2016	2014–2017	Not detected	Not detected
Pacific Lamprey	Upstream Elwha Dam	Aug 2014	2014–2017	Mar 2013	Smolt Trap[e]
	Upstream Glines Canyon Dam	Sep 2014	2014–2017	Jul 2017	Radiotelemetry[f]

[a]McHenry redd survey reports.
[b]Geffre radiotelemetry report.
[c]Unpublished NPS files.
[d]Weekly netting to estimate species composition that is conducted by researchers running sonar equipment for adult escapement estimates.
[e]Smolt trap run by lower Elwha Klallam Tribe.
[f]Rebecca Paradis, Lower Elwha Klallam Tribe, unpublished radiotelemetry study.

taxa in Figure S1) revealed an effect of distance from the mouth of the river, as downstream sites in LE and ME had a higher probability of eDNA detections than upstream sites in UE for most species in the years following dam removal. Our temporal eDNA surveys, in conjunction with traditional fisheries techniques (Table 4), helped confirm that migratory fish were ascending the watershed and passing the former locations of both dams and added additional information showing that the spatial extent and temporal dynamics of movements into the watershed differed by species.

The timing and spatial extent of recolonization differed among migratory Pacific Salmon. For Chinook Salmon, the most abundant anadromous salmonid following dam removal, an increasing probability of eDNA detections farther upstream was apparent as fish continued to expand their spatial extent upstream into the watershed. Their probability of occurrence in upper portions of the watershed by the 4th year (2017) was greater than the first 3 years after dam removal (Figure 3). The first detection of Chinook Salmon in UE was rapid, as an individual was first seen immediately upstream of the former Glines Canyon Dam within days of the final blast removing the last remnant portion of the dam. Three weeks later, we detected Chinook salmon eDNA at Elkhorn, over 20 rkm upstream.

Other species like Chum Salmon and Pink Salmon, which were in critically low numbers prior to and following dam removal, showed

a more limited and less persistent spatial extent into areas upstream of the former Glines Canyon Dam site. By the fourth year of sampling in 2017, both species had been detected upstream of both former dams, but with trace detections and limited spatial extent. This contrasting pattern of dispersal into the upper portions of the watershed between Chinook and Pink/Chum was predicted based on intrinsic potential modeling prior to dam removal, life-history characteristics, and migration timings, as well as the size of and distance to source populations (Pess et al., 2008).

Coho Salmon also responded rapidly to dam removal, becoming established in Indian Creek in the early stages of the project due to assisted relocations of adults from downstream areas that were being impacted by high sediment concentrations (Liermann et al., 2017). This assisted recolonization continued during the dam removal period and helped Coho salmon become established in specific areas of the ME. However, even with this effort, we did not detect Coho Salmon with as much frequency in the upper portions of the watershed beyond the relocation areas, a result consistent with 2018 and 2019 whole river snorkel surveys (the authors, unpublished data, using the technique described in Brenkman et al., 2012).

Sockeye Salmon were hypothesized to return as fish recolonized Lake Sutherland (Hansen, Gardner, Beauchamp, Paradis, &

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Quinn, 2016), the historical spawning and rearing area of this species in the watershed. Located between the dams, Lake Sutherland is connected to the Elwha via Indian Creek. During our sampling after dam removal, we detected Sockeye Salmon eDNA at low frequency throughout the mainstem of the river, including in areas far upstream of Indian Creek's confluence with the Elwha River. This was a counterintuitive result, because the presumption was that Sockeye Salmon would be limited to spawning and rearing in Lake Sutherland and Indian Creek. Subsequent genetic testing of tissue samples from adult Sockeye Salmon captured during the same period as this effort suggested that most Sockeye Salmon were strays from other populations, including the West Coast of Vancouver Island and Southwest Alaska (Tom Quinn et al., unpublished data in review).

The use of eDNA was effective for documenting Lamprey in the Elwha River and tributaries, a result seen in other watersheds of Puget Sound (Ostberg et al., 2018). As barriers are removed, Pacific Lamprey can quickly move in to occupy and recolonize habitats upstream, as seen in the removal of the Condit Dam on the White Salmon River in Washington State (Jolly, Silver, Harris, & Whitesel, 2018). This appears to be happening in the Elwha River, as adult and juvenile Pacific Lamprey were detected upstream of the Elwha Dam in Indian Creek following dam removal (Moser & Paradis, 2017). Additional studies will need to verify spawning activities upstream of the Glines Canyon Dam where we detected Pacific Lamprey eDNA. The detection of a previously undocumented taxa (*Lampetra* spp.) also occurred in our study, but at extremely low levels of eDNA detection. Despite repeated electrofishing with lamprey-specific gear and genetic testing from lamprey tissues that have been captured in the Elwha, *Lampetra* spp. have never been documented in the watershed (Rebecca Paradis, Lower Elwha Klallam Tribe, personal communication). Ten years prior to the start of eDNA sampling, a single juvenile lamprey was captured by the senior author during invertebrate sampling near the Hayes River (rkm 51) as part of another project (Morley, Duda, Coe, Kloehn, & McHenry, 2008), yet no species verification was conducted. It is possible that this individual was *Lampetra* spp. or possibly part of a Pacific Lamprey population that had become landlocked after the dams were constructed, a phenomenon that has recently been described for another Pacific Lamprey population in Oregon (Larson, Helstab, Docker, Bangs, & Clemens, 2020). Additional surveys using other field techniques (e.g., those that would allow field identification and genetic confirmation) are required to verify what would be the first record of the *Lampetra* in the watershed.

Our study also showed how Brook Trout, a non-native species restricted to isolated areas between the dams prior to dam removal (Brenkman et al., 2008), apparently dispersed downstream of the former Elwha Dam into LE but had yet to disperse upstream into UE in an appreciable manner (i.e., only trace detections in the Mills Reservoir floodplain, but occurring in three consecutive years). A concern prior to dam removal was the potential for Brook Trout to move into newly available areas upstream of Glines Canyon Dam. It is unclear whether downstream detections in LE were from Brook

Trout eDNA being transported from extant upstream populations in ME, or whether new populations have become established in downstream areas. Were the former to be true, it would mean that eDNA would have been transported at least 7,500 m, the distance between a large source population in Indian Creek (rkm 12.2) to the downstream detection site at Bike Bridge (rkm 4.7).

A large body of studies have shown that eDNA signals typically persist for 100s rather than 1000s of meters downstream from a source, but this depends upon hydrological conditions, density of target species, and potentially numerous other factors (e.g., Spear, Groves, Williams, & Waits, 2015; Tillotson et al., 2018; Wilcox et al., 2016). Using caged fish in otherwise fishless streams, Jane et al. (2015) showed that eDNA was detectable at their downstream most sampling location 237 m downstream, whereas Civade et al. (2016) detected lentic taxa found in an upstream lake in river samples 2,000–3,000 m downstream. Pilliod et al. (2014) showed that salamander eDNA could be detected 450 m from the source of caged animals, while Wacker et al. (2019) detected eDNA from western pearlshell mussels (*Margaritifera falcata*) 1,700 m downstream from source populations. Thus, it appears likely that downstream Brook Trout eDNA detections were from local sources rather than from genetic material transported downstream. Downstream dispersal of Brook Trout could be voluntary or a result of a competitive disadvantage with recolonizing Coho Salmon (Thornton, Duda, & Quinn, 2016). Additional electrofishing surveys in downstream areas of the Elwha are needed to confirm whether Brook Trout have truly dispersed and become established metapopulations.

5.1 | Benefits and limitations of using eDNA to assess recolonization following dam removal

Our study is among a growing body of literature demonstrating that eDNA can be used to monitor the presence, occurrence, and spatial extent of fish populations (Laramie, Pilliod, & Goldberg., 2015; Matter, Falke, López, & Savereide, 2018; Ostberg et al., 2019; Rees et al., 2014; Wilcox et al., 2013) and to assess river connectivity for migratory fishes (Yamanaka & Minamoto, 2016). In the case of the Elwha River, we used eDNA to assess the presence of fish in upstream areas of the watershed that were previously inaccessible due to the presence of the dams, which provides evidence that key objectives for the dam removal project (i.e., fish passage, restoring connectivity) are being met for several focal species (Peters et al., 2014). With a single technique and a network of sampling locations, we were able to assess the presence of different species with a range of life histories and ecological needs. In a management context, there were management-intensive (i.e., species listed as threatened under the U.S. Endangered Species Act), rare, non-native, and migratory/resident species present in the basin.

Results from contemporaneous field studies using other fisheries techniques in the Elwha verify many of the observations we made using eDNA (Table 4). Ongoing spawner (McHenry et al.,

2016; McHenry, Pess, Anderson, & Hugunin, 2017; McHenry, Pess, & Anderson, 2018), snorkel, electrofishing (Liermann et al., 2017), and radiotelemetry studies (Brenkman, Peters, Tabor, Geffre, & Sutton, 2019; Geffre, Brenkman, Peters, & Crain, 2018) have also shown migratory species passing both former dams, as well as provided information on the maximum extent of recolonization for some species (Table 4). The first detections of Sockeye Salmon, Chum Salmon, Pink Salmon, and Pacific Lamprey upstream of the former Glines Canyon Dam site were from eDNA results, while other traditional methods that were being regularly employed detected them later or not at all (Table 4). The high levels of Brook Trout eDNA detections were also consistent with results from smolt trapping in Indian Creek.

However, the ability to answer additional questions with the more traditional techniques that produced additional types of data was apparent. For example, Liermann et al. (2017) describe Coho Salmon spawning and rearing in the ME tributaries of Little River and Indian Creek. Although Coho Salmon eDNA detection in these two tributaries was similar across all years (Figure 2), Liermann et al. (2017) documented that while the numbers of adults and redds produced were similar between these tributaries, Indian Creek on average produced about 4 times more smolts. Previous studies have shown that eDNA concentration and species abundance may be correlated (e.g., Levi et al., 2019; Tillotson et al., 2018), but we did not quantify eDNA concentration for this study. Future studies using eDNA could compare eDNA concentration, adjusted for stream flow, between these tributaries during both the adult migration period (fall) and the juvenile outmigration period (spring) to test whether differences in stream productivity could be detected using eDNA. Because our use of eDNA only estimated presence or nondetection of targeted species, it provided limited information for describing the dynamics of how each species responded to dam removal.

Another limitation of our application of eDNA was that for some species, we were unable to document successful migrations past the former dams because our assays do not differentiate between anadromous and resident forms. Both Bull Trout and Rainbow Trout occurred throughout the watershed prior to dam removal, so detecting any patterns of migratory or metapopulation dynamics due to dam removal using eDNA was not possible. Yet, both species had significant responses to dam removal, including the evolution of life-history diversity and the resumption of anadromy (Brenkman et al., 2019; Quinn, Bond, Brenkman, Paradis, & Peters, 2017). For example, within river migrations, including passage past each former dam and maximum extent of adfluvial migrations were documented with radiotelemetry for Bull Trout, Rainbow Trout/Steelhead, and Pacific Lamprey (Brenkman et al., 2019; R. Paradis, Lower Elwha Klallam Tribe, unpublished data). Further, radiotelemetry also documented long-distance movements, with some individual Bull Trout traveling over 130 river kilometers from the estuary to the headwaters and back again after receiving a transmitter (Brenkman et al., 2019) following dam removal.

We also observed a few instances of trace detections of salmon eDNA upstream of anadromous barriers in our tributary sites. Our intention of including these above barrier sites was to serve as a field-based false-positive control. In each of these cases, there was a single PCR detection from one water sample on a single sampling occasion. Two possible explanations account for the false-positive detections in these areas where salmon do not have access. The first and perhaps more likely is field or laboratory contamination, either from collecting the water sample and having transference of eDNA material into the water sample or processing the sample in the laboratory and transferring eDNA material into sample or real-time PCRs. The other is allochthonous contamination, which would happen if salmon eDNA was transferred from a downstream source to an above barrier site. Allochthonous contamination could occur via multiple bird species (e.g., common merganser, harlequin duck, American dipper, bald eagle, gulls) that either reside in water with salmon or actively feed on salmon. In a carcass addition experiment, Morley et al. (2016) seeded a 100-m portion of an Elwha side channel for a food web study with 0.75 kg/m^2 of salmon carcasses that were completely removed within 1 week by animal scavengers. Other cases could occur when mammalian scavengers like racoon or bears feed on salmon carcasses. It is not unreasonable to assume that these birds or mammals could be vectors of genetic material, either from attaching to their bodies or passing through their gut (Merkes, McCalla, Jensen, Gaikowski, & Amberg, 2014).

6 | CONCLUSION

We found that eDNA was an effective tool to track the recolonization and redistribution dynamics of fish species following dam removal. With relatively modest costs compared to other fisheries techniques, we were able to conduct a long-term study over a 40-month period, revisiting sites across seasons and years to track the changing spatial distribution of species into the watershed. Multiscale occupancy modeling based upon the adult migration period showed differing dynamics for different species, which matched predictions for some species like Chinook, Coho, Pink, and Chum salmon. It also revealed unexpected results, like Sockeye Salmon that were expected to have a distribution limited to a lake-fed subbasin that were detected far upstream. We also were able to detect eDNA from non-native Brook Trout into downstream areas where it had been rare prior to dam removal. The use of eDNA for monitoring the presence of targeted species and tracking their movement into portions of watersheds following barrier removal projects is an effective application of this emerging monitoring tool.

AUTHOR CONTRIBUTION

JJD, COO, and MSH conceived and designed the study. DMC, MSH, and JJD performed fieldwork and data collection. JJD and COO performed data analysis. All authors contributed to drafting and editing the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at the U.S. Geological Survey data repository: https://doi.org/10.5066/P96R5Q0M.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.